AKT Isoform-specific Monoclonal Antibodies

Rockland Immunochemicals, Inc.

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Rockland AKT Isoform Specific Monoclonal Antibodies

- AKT (Protein Kinase B or PKB), is a serine/threonine-specific protein kinase with known key involvement in glucose metabolism, apoptosis, cell proliferation, transcription and cell migration.
- More recently, AKT activation is associated with tumor cell survival, proliferation, and invasiveness.
- AKT inhibitors are the subject of ongoing clinical trails for the treatment of cancer.
- Isoforms of AKT are found to be distinct with regard to tissue expression, pathway activation and inhibitor sensitivity.
- Rockland has created a panel of monoclonal antibodies to AKT isoforms suitable for probing lysates and pharmacodynamic studies.

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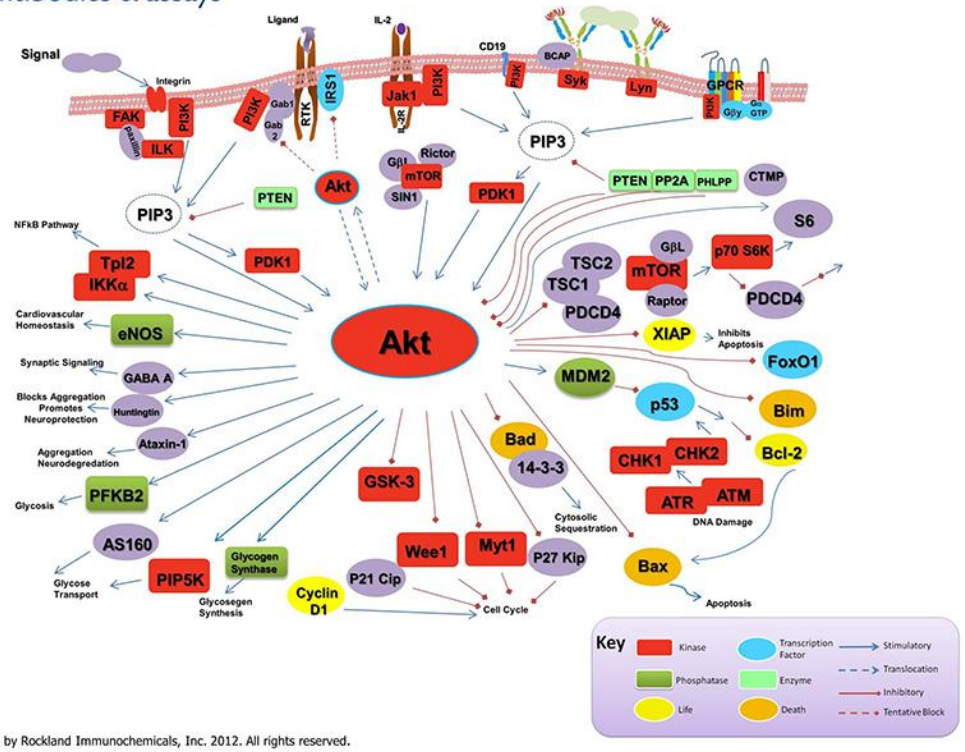
Rockland produces antibodies to numerous AKT pathway targets



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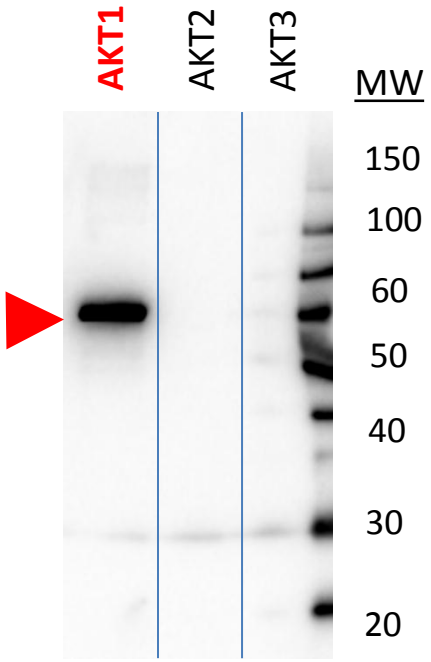
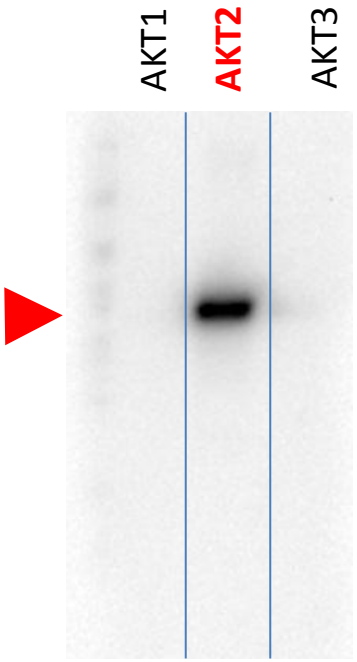
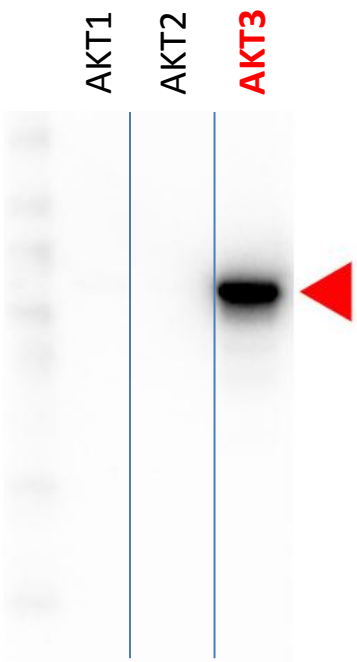
Western Blot of AKT Isoform Specific Antibodies



Anti-AKT1 Monoclonal Antibody - 200-301-I51



Anti-AKT2 Monoclonal Antibody - 200-501-E71



Anti- AKT3 Monoclonal Antibody - 200-301-E75

Western Blot of AKT isoform specific antibodies: 50 ng of recombinant GST-AKT1, -AKT2 and -AKT3 proteins were separated by SDS PAGE (4-20% gel) and transferred onto 0.2 µm nitrocellulose. The membrane was blocked with Blocking Buffer (p/n MB-070) for 1 h at room temperature. Mouse anti-AKT1 clone 14E5A2B2 (p/n 200-301-I51) , Rat anti-AKT2 clone 16G11A7 (p/n 200-501-E71) and Mouse anti-AKT3 clone 25F6F6D8 (p/n 200-301-E75) were applied at a dilution of 1 µm/mL in Blocking Buffer and incubated for 16 h at 4°C. Rabbit anti-Mouse IgG HRP (p/n 610-403-C46) or Goat anti-Rat IgG HRP (p/n 612-103-120) secondary antibodies were applied at 0.05 µg/mL in Blocking Buffer and incubated for 1 h at room temperature followed by detection with FemtoMax™ chemiluminescent reagent (p/n FEMTOMAX-110). Arrowheads indicate the position of each AKT isoform in blots.

Lane 1: GST-AKT1; Lane 2: GST-AKT2; Lane 3 GST-AKT3. The expected size for all recombinant GST-AKT isoforms is 85 kDa.

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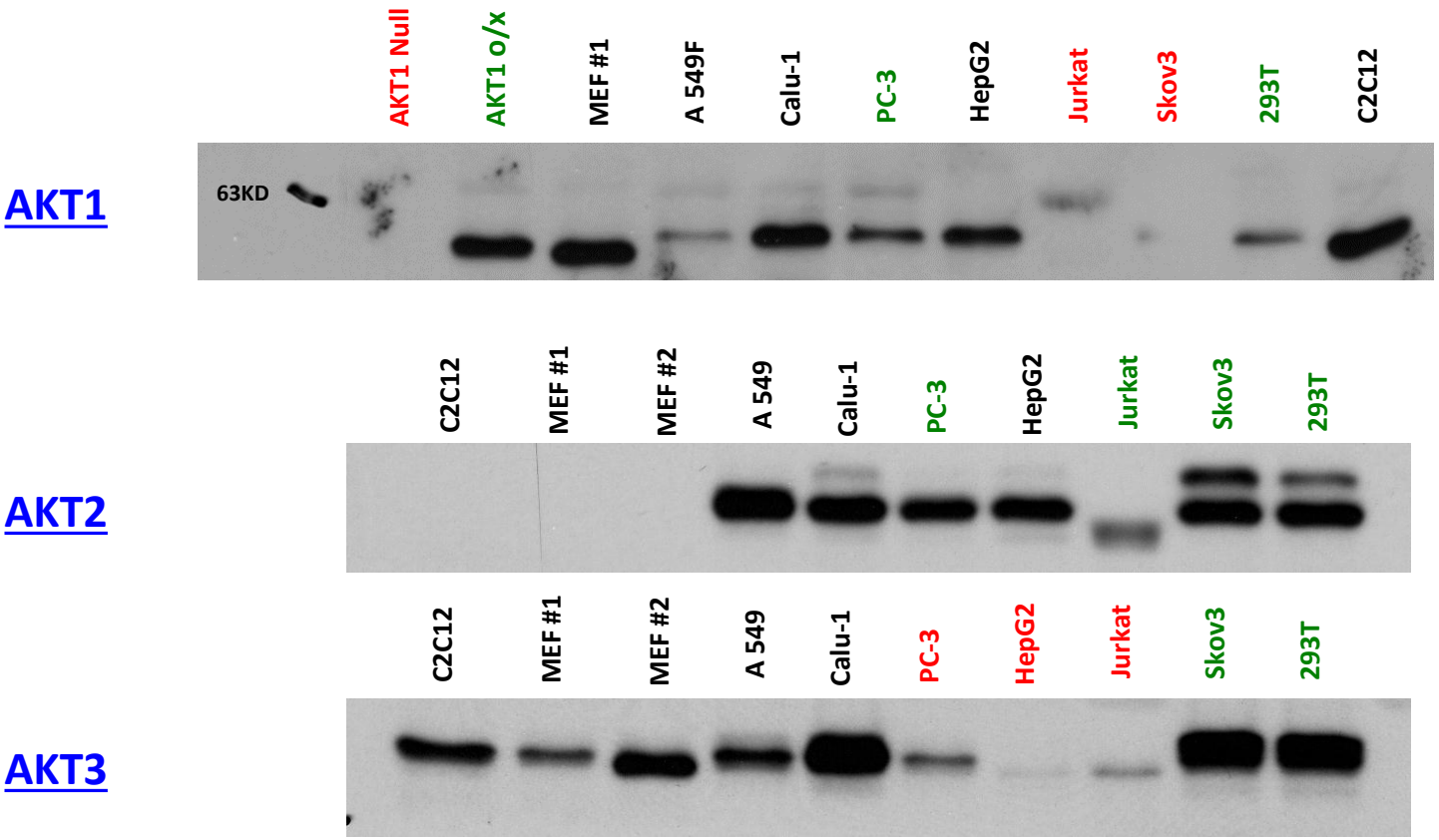
mAbs Identify Specific AKT Isoforms in Cell Lines



(Unpublished data provided by Dr. Yuefeng Tang, Dave Guertin Lab. U. Mass)

Western Blot using a Panel of Cell Lines: Whole cell lysates (20 µg) prepared from a panel of cell lines with differential expression levels of AKT isoforms were prepared for separation by SDS PAGE (4-20%) and transfer onto 0.2 µm nitrocellulose membrane. After blocking with Blocking Buffer (MB-070) for 1 h at room temperature, respective AKT isoform specific antibodies were applied at dilution 1 µg/mL in Blocking Buffer and incubated overnight at 4 °C. Signal was processed as before. Cell lines *predicted* to express specific AKT isoforms are printed in green. Cell lines *predicted* not to express a specific AKT isoform are printed in red.

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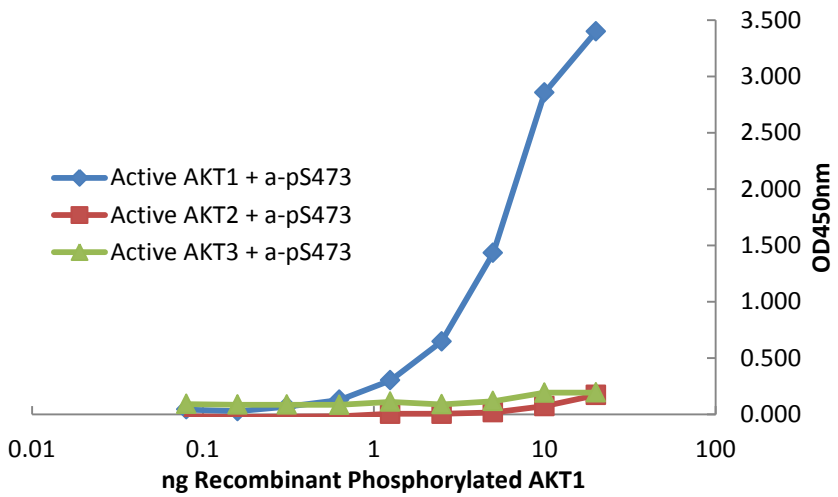
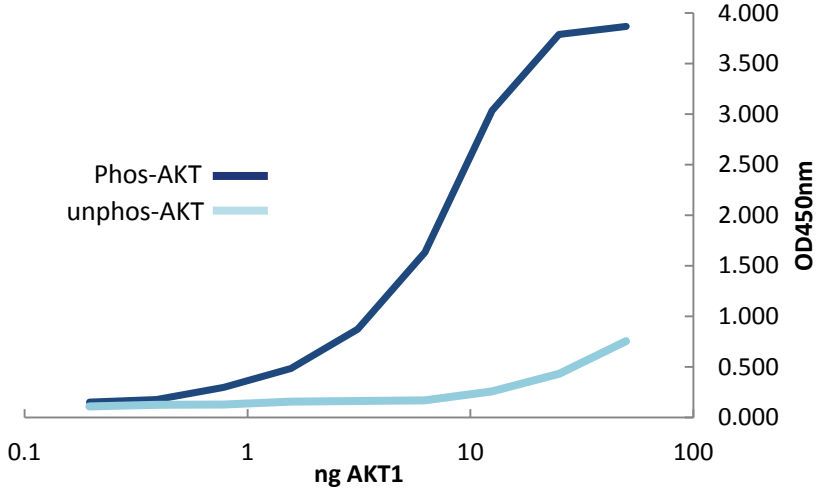
AKT1 Isoform Specific Antibody

Capture ELISA against phosphorylated AKT1, AKT2, and AKT3



Capture ELISA against phosphorylated and non-phosphorylated AKT1



p/n 200-301-I51 Anti-AKT1 mAb 14E5.A2

LEFT PANEL: Plate was coated with monoclonal anti-AKT1 antibody (capture antibody) followed by incubation with activated recombinant AKT1, AKT2, AKT3 proteins. Binding was detected with biotinylated mAb anti-AKT pS473.

RIGHT PANEL: Plate was coated with monoclonal anti-AKT1 antibody (capture antibody) followed by incubation with recombinant AKT1 or phosphorylated AKT1 proteins. Binding was detected with biotinylated monoclonal anti-AKT pS473.

SUMMARY: Anti-AKT1 monoclonal antibody is specific to recombinant AKT1 protein and not isoform 2 and 3. Anti-AKT1 isoform specific antibody and anti-AKT-pS473 (p/n 200-301-268) are suitable antibody pairs for pharmacodynamic assays. Unpublished PD assay data not shown.

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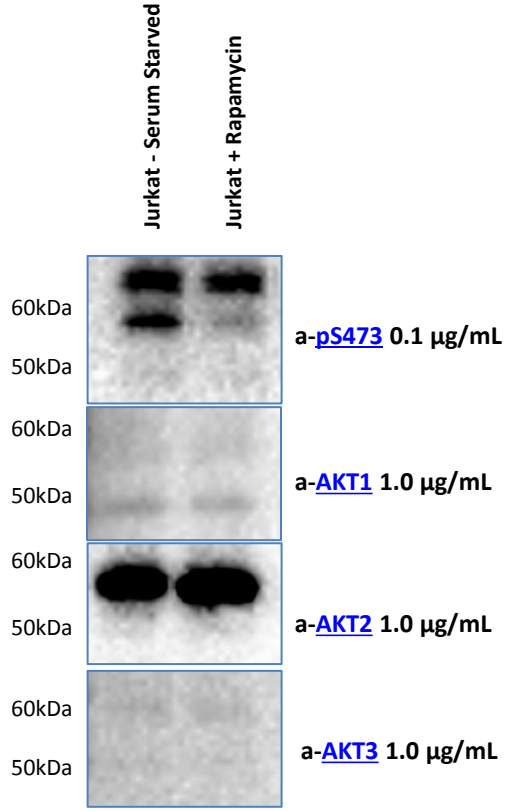
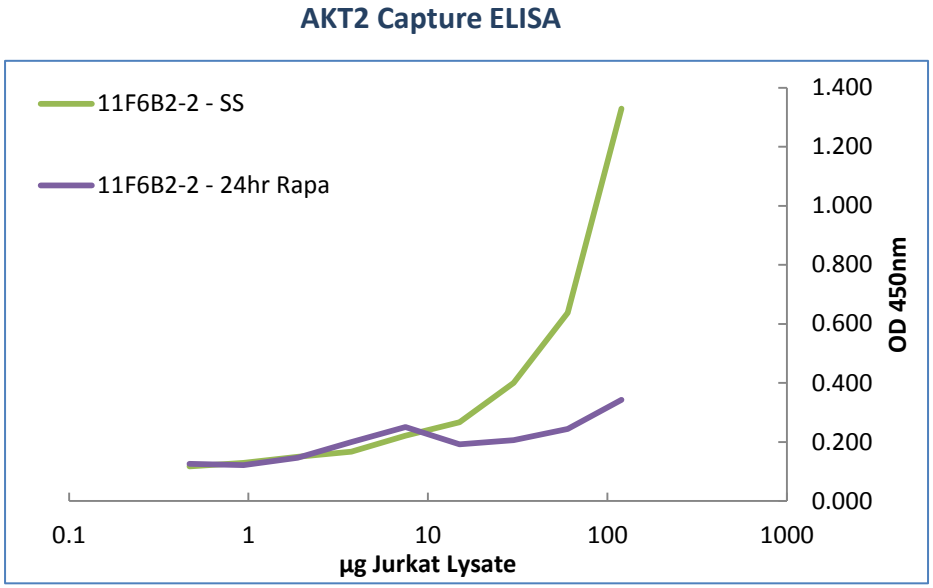
AKT2 Isoform Specific Antibody



p/n 200-501-E71 Anti-AKT2 mAb 16G11.A7

Western Blot of Jurkat Cell Lysates: Whole cell lysates were prepared from cultured Jurkat cells serum starved for 24 h (left lane), or treated with Rapamycin for 24 h (right lane). Various AKT antibodies were used to probe the lysates (20 µg) as indicated. Jurkat cells express pAKT2 constitutively with and without the presence of Rapamycin, however the phosphorylation status of AKT2 is greatly reduced when incubated with the inhibitor. Note - the anti- pS473 band at 70 kDa is known cross-reactivity with S6K.



AKT2 ELISA of Drug Treated Cell Lysates: AKT2 isoform specific antibody (cMab) paired with pS473 antibody (dMab) shows greater than 10:1 S/N when serum starved Jurkat lysates (120 µg) are applied. Little to no reactivity is observed after rapamycin treatment. Similar results are seen with other well characterized cell lines and other drug candidates (unpublished data not shown).

SUMMARY: Antibodies confirm that Jurkat cells predominantly contain AKT2. Anti-AKT2 isoform specific antibody and anti-AKT-pS473 antibody are suitable antibody pairs for lysate studies and pharmacodynamic assays.

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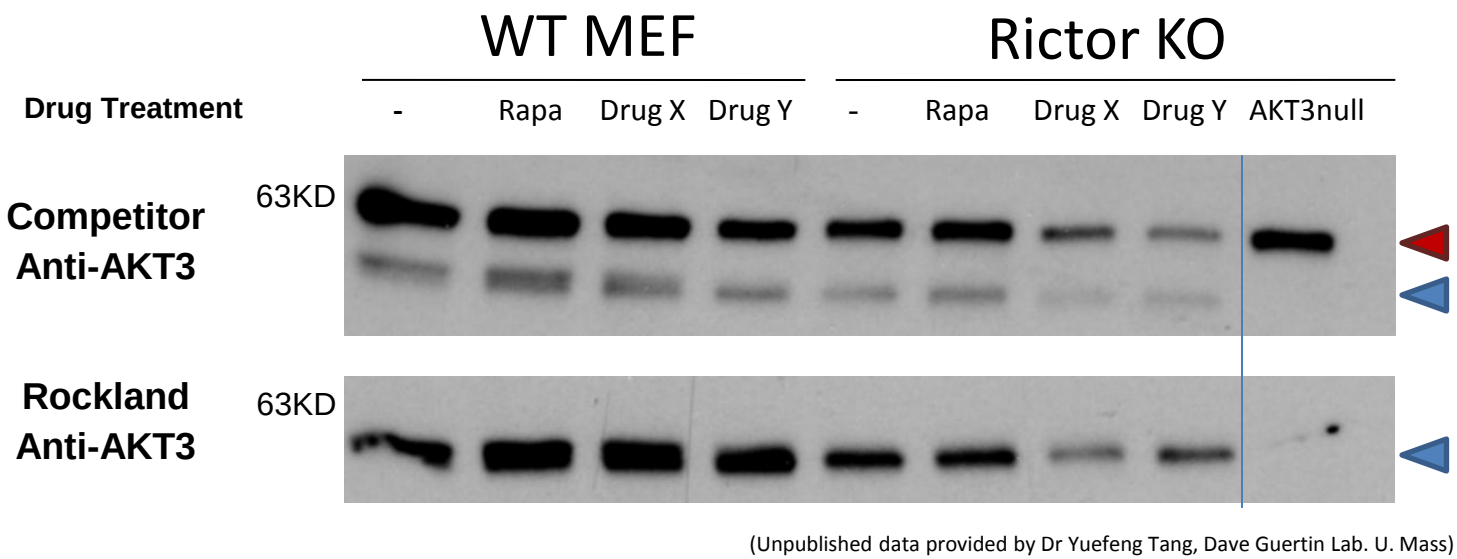
AKT3 Isoform Specific Antibody



(Unpublished data provided by Dr Yuefeng Tang, Dave Guertin Lab. U. Mass)

p/n 200-301-E75 Anti-AKT3 mAb 25F6F6D8

Western Blot Comparative Study: Wild type mouse embryonic fibroblast (MEF) cells and cell lines created from Rictor knockout mice were treated with rapamycin and two other drugs (unpublished). Untreated cell lysates were similarly processed along with cells from AKT3 null mice. Rockland's anti-AKT3 antibody (bottom panel) detects AKT3 (blue arrowhead) in wt MEFs. While rapamycin appears to have little affect on AKT3 levels in cells from Rictor knockouts, the two other drugs used in this study show a pronounced effect. By comparison, a competitor's anti-AKT3 antibody (top panel) appears to incorrectly recognize a 63 kDa band (red arrowhead) in MEFs and AKT3 null cells.

SUMMARY: Anti-AKT3 isoform specific antibody correctly identifies AKT3 in a panel of lysates derived from wild type, knock out and null cells.

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Rockland AKT antibodies

- Rockland AKT isoform specific monoclonal antibodies represent novel tools for AKT signal pathway research. These reagents are suitable for use in expression studies and pharmacodynamic assays.
- Successful use in both western blot and ELISA applications suggests platform independence.
- Rockland AKT isoform specific monoclonal antibodies are validated by multiple assays including western blot, ELISA and immunoprecipitation.

Catalog Number	Product Name	Clone ID	Host Animal	Subclass
200-301-I51	Anti-AKT1 Monoclonal Antibody	14E5.A2	Mouse	IgG1 kappa
200-501-E71	Anti-AKT2 Monoclonal Antibody	16G11.A7	Rat	IgG2a kappa
200-301-E75	Anti-AKT3 Monoclonal Antibody	25F6.F6.D8	Mouse	IgG1 kappa

Other AKT antibodies				
200-301-268	Anti-AKT pS473 Monoclonal Antibody	17F6.B11	Mouse	IgG1 kappa
200-301-269	Anti-AKT pT308 Monoclonal Antibody	18F3.H11	Mouse	IgG1 kappa
200-301-401	Anti-AKT Monoclonal Antibody	18F10.E5	Mouse	IgG1 kappa

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